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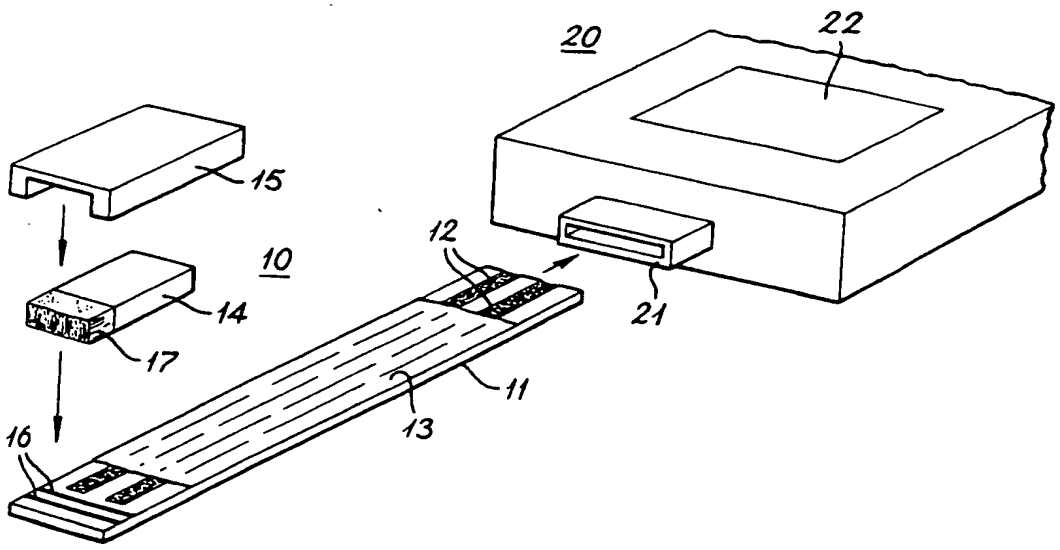

(57) Abstract

A disposable reactor (10) is provided for use with a co-operating instrument (20) to form an analytical device. The reactor includes a body of capillary material (14) housed in an enclosure (11, 15) having an opening for application of liquid to the body. At least one reagent (16) is immobilised in the enclosure. Also an electrode assembly (12, 12) passes through the enclosure, with one portion of the assembly extending within the enclosure to engage the capillary body and another portion of the assembly extending outside the enclosure, remotely from the enclosure opening, for connection with the associated instrument.

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ANALYTICAL DEVICES

Analytical devices for extralaboratory use are coming into greatly increased usage and they are, at the same time, becoming available in an increasing variety. This is particularly true of such devices related to medicine, but not exclusively so.

These devices are preferably of a self-contained form, typically requiring only the application of a sample of a body fluid such as urine or blood, for example. However, the devices are mostly of a qualitative or semi-qualitative form requiring some ability on the part of the user to interpret the analytical result. Because the user is commonly not skilled for this last purpose, and in fact is often a lay individual, the user-dependent nature of the devices makes them less than completely satisfactory.

This situation is reflected by the fact that a trend can be seen in favour of devices of a quantitative form and the invention concerns such devices.

The present invention particularly concerns quantitative devices of a two-part form involving a reusable instrument co-operable with disposable analytical reactors. More particularly, the invention concerns such two-part devices in which the reactor involves a body of capillary material carrying at least one immobilised reagent and engaging an electrode assembly connectable with an associated instrument. In use, a sample for analysis, and liquid to mobilise the reagent or reagents, are applied to the capillary body and the resultant reaction influences the electrode assembly in a manner dependent on the sample to provide a related quantitative output from the instrument. Such a device and use offers several advantages but, as so far proposed, also suffers from some disadvantages associated with the capillary body.

One of these disadvantages is that, unless the capillary body is compact, reagent can be mobilised in an undesirable manner to create areas of significantly different reagent concentrations.

Another disadvantage is that, if the mobilising liquid is applied by dipping the body into a liquid reservoir, which is attractive for its simplicity, reagent can be leached from the body into the reservoir and impair the desired reaction. A further disadvantage is that the liquid contents of the body can be adversely affected by movement of the body such as occur, for example, with dipping.

An object of the present invention is to reduce these disadvantages and, to this end, the invention provides a disposable reactor for use with a co-operable instrument to form a quantitative analytical device, the reactor comprising a body of capillary material, a liquid-impermeable enclosure housing the capillary body and having an opening for application of liquid to the body, at least one reagent immobilised within the enclosure, and an electrode assembly passing through the enclosure, with one portion of the assembly extending within the enclosure to engage the capillary body and another portion of the assembly extending outside the enclosure, remotely from the enclosure opening, for connection with the associated instrument.

A reagent can be immobilised in the enclosure by impregnation or other incorporation in the capillary body and/or by deposition on or other incorporation in the interior of the enclosure.

Preferably the enclosure and the housed capillary body, are of an elongate form with the opening at one end of the enclosure and the electrode assembly passing through the opposite end. Such a form suitably involves a strip base for the enclosure, an electrode assembly extending longitudinally along one side face of the base, a capillary body also of strip form and located on the base as well as one end portion, but not the other, of the electrode assembly, and an enclosure cover extending transversely over the body and connected with the base while leaving the electrode assembly other end portion exposed. The electrode assembly can in fact be directly covered with a layer of electrically insulating material over much of its length provided

that it is exposed towards its ends respectively for operable engagement with the capillary body and an associated instrument.

The above-described and other facets of the invention are clarified by the following further description, given by way of example, of the accompanying drawings in which:-

Figure 1 schematically illustrates in exploded manner one form of a reactor according to the invention together with an associated instrument.

Figure 2 illustrates the results obtained for one form of ana tical assay effected with a particular embodiment of the reactor of Figure 1,

Figure 3 illustrates the reactions involved in another form of assay,

Figure 4 illustrates results obtained for the assay of Figure 3 with another reactor embodiment, and

Figures 5 and 6 respectively illustrate yet another assay and results associated with a further reactor embodiment.

Figure 1 the reactor and associated instrument are denoted generally at 10 and 20.

The reactor 10 has a base 11 of strip form made of material which is liquid impermeable and electrically insulating. An electrode assembly consisting of two mutually spaced parallel electrodes 12 are mounted longitudinally on one face of the base and have an intermediate portion of their length covered by a layer 13 of further impermeable insulating material to leave the electrodes exposed at their extremities. A body 14 of capillary material is, in turn, located to cover the exposed electrodes at one extremity. The body 14 is itself covered by a further layer 15 of impermeable insulating material which extends transversely round the body to connect with the base, while leaving exposed the end of the body remote from layer 13. Lastly, at least one reagent 16 is deposited on part of the base covered by the body.

In several different embodiments of this reactor form constructed during initial development of the invention, the base

was made of polyvinylchloride (PVC). The electrodes were applied by screen coating with one, the working electrode, being of carbon and the other, the reference or counter electrode, being of silver, surface treated to provide a coating of silver chloride. The capillary body was made of 0.5 mm thick PVA foam (Grade PRO/800; Prosthex Ltd., Surrey, England). The layers 13 and 15 were made of adhesive electrical insulating (PVC) tape and polymethylmethacrylate (Perspex), respectively. Reagent compositions differed between the embodiments for the purposes of respectively different analyses.

The instrument 20 can be of any suitable form adapted for cooperation with the reactor. Clearly it will have a socket 21 or other connector for mutual engagement or other working cooperation with the exposed electrode assembly portion of the reactor. Typically the instrument includes electronic components operable to respond to a representative potential difference set up between the reactor electrodes under the influence of the analytical reaction and to indicate that difference, or a resultant current flow, as a quantified output at a visual display 22. The instrument can also include components operable to render the former fully operable in response to use of a reactor connected therewith, such as by reaction to liquid application. In addition, other components can effect temperature compensation, switch between a range of operational modes in response to differential coding incorporated in reactors of different analytical type, and effect other useful functions.

Turning to the more specific detail of embodiments of the invention used for three different assays of analytical significance, materials used were as follows:- Alcohol dehydrogenase (EC 1.1.1.1.), diaphorase (EC 1.8.1.4) type II-L, glucose oxidase (EC 1.1.3.4) type X, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) type VII, hexokinase (EC 2.7.1.1) type III, adenosine triphosphate (dipotassium salt), β-nicotinamide adenine dinucleotide (NAD) sodium salt and β-nicotinamide adenine dinucleotide phosphate (NADP) sodium

salt, from the Sigma Chemical Co. Poole, Dorset, England. Diaphorase (EC 1.8.1.4) type II from Boehringer Mannheim UK, Lewes, East Sussex, England. Aluminium nitrate nonahydrate (99.9999%), magnesium chloride hexahydrate (99.995%) and 1,1'-dimethyl-ferrocene from the Aldrich Chemical Co., Gillingham, Dorset, England. Imidazole (extra pure) from BDH Chemical Co., Poole, Dorset, England. Polyvinylpyrrolidone (Av. Mr. 10 kda.) from Fluka Chemicals Ltd., Glossop, Derbyshire, England. Potassium ferricyanide from Fissons Ltd., Loughborough, Leicestershire, England. 1:1 solutions of polyvinylpyrrolidone were prepared by adding one part by weight polyvinylpyrrolidone to one part by weight of buffer solution and mixing until a smooth paste was formed.

Assay 1. This assay was carried out for glucose. The carbon working electrode of the reactor was doped with 1,1'-dimethyl-ferrocene and had the enzyme glucose oxidase immobilised on to it. Glucose solutions were made up in phosphate buffered saline and allowed to stand overnight. Assays were carried out by touching the surface of the glucose solution with the opening end of the reactor. This caused the solution to wick up the capillary body and come into contact with the electrodes to provide a response which was almost instantaneous. This response was in the form of a potential difference across the reactor and this was applied to an instrument in the form of a 4700 μF capacitor deployed to integrate the relevant voltage for two minutes. At the end of this time the voltage across the capacitor was determined using a multimeter. This was plotted against glucose concentration in the sample solution to give a result indicated by Figure 2.

Such an embodiment can be useful to carry out glucose and other determinations in physiological fluids like blood. For this purpose it can be appropriate to incorporate a filter in the reactor enclosure, between the capillary body and opening, as indicated at 17 in Figure 1. This filter is effective to remove unwanted materials such as red blood cells from the incoming

sample. Also such an embodiment may usefully be integrated with a lance or other implement useful in providing a blood or other sample. Similar considerations might also apply to other materials for analysis, such as certain foods. These, for example jam, are often viscous and not amenable to normal analytical techniques. However, with the incorporation of a filter into the reactor, it is possible selectively to isolate the free-flowing component and carry out an assay on this.

Assay 2. This assay was carried out for ethanol. It is described here as an example of an analytical reaction that involves a soluble co-enzyme (NAD). For this purpose, the reactor embodiment involved the enzymes alcohol dehydrogenase (10 mg ml^{-1}) and diaphorase (2 mg ml^{-1}), the co-enzyme NAD (10 mg ml^{-1}), and the electron acceptor potassium ferricyanide (40 mg ml^{-1}), immobilised in 1:1 polyvinyl-pyrrolidone (made up with 0.2 M pyrophosphate buffer, pH 9.0) on the reactor base. When an aqueous solution of ethanol was drawn into the reactor, the reagents were dissolved. Ferricyanide is reduced to ferrocyanide as shown in Figure 3. This was detected electrochemically at the working electrode. Again, the voltage resulting from the analytical reaction was used to charge up a capacitor, the voltage across the capacitor after two minutes was plotted against the concentration of ethanol, and the resultant operational characteristic is shown in Figure 4.

Assay 3. This assay was carried out for aluminium and it is described here as an example of an analytical reaction that involves enzyme inhibition. For this purpose the reactor embodiment involved the enzymes hexokinase (40 µg ml^{-1}), diaphorase (2 mg ml^{-1}), and glucose 6-phosphate-dehydrogenase (20 µl ml^{-1}) and the co-enzyme NADP (10 mg ml^{-1}), immobilised in 1:1 polyvinylpyrrolidone (made up in 1.25 M imidazole buffer, pH 6.9, containing 1 mM magnesium chloride), on the reactor base. Also, the substrates adenosine triphosphate (15 mg ml^{-1}) and glucose (50 mg ml^{-1}) and the electron acceptor ferricyanide (80 mg ml^{-1}) were immobilised in 1:1 polyvinylpyrrolidone (made

up in 1.25 M imidazole buffer, pH 6.9, that contained 1 mM magnesium chloride) on the inner face of the enclosure. When such a reactor draws up an acidic aqueous solution of aluminium nitrate (made by dissolving aluminium nitrate in 10 mM nitric acid) the reagents dissolve in it and ferricyanide is reduced to ferrocyanide as shown in Figure 5. The reaction was allowed to proceed for five minutes. Ferrocyanide was detected electrochemically with the resultant voltage being applied to charge up a capacitor. The integrated capacitor voltage after one minute was plotted against the concentration of aluminium to give a characteristic as shown in Figure 6.

While the invention has been described with more particular reference to the illustrated reactor form and assay-specific embodiments thereof, it is clearly open to considerable variation within the broader introductory description.

For example, the reactor can accommodate a sequence of reactions involving the same sample, with an associated instrument giving individual and/or, if appropriate, composite quantitative results for the respective analytical reactions. For this purpose the reactor base can have the appropriate reagents applied thereto as respective transverse bands in a successively spaced assay along the base so that the reagents are mobilised in sequence as liquid is drawn into the capillary body. Alternatively, or in addition, plural capillary body channels leading to a common site can be provided, as proposed in Patent Specification WO 90/11519.

In another example, an alternative reactor form involves a compressed or otherwise liquid-expansible capillary body. Such a body can expand when activated by the application of liquid to trap a thin film of the liquid against the associated electrodes. Also, such an expansion can be used to close off the enclosure adjacent the opening to prevent, or at least reduce, outward leaching. Similarly, it is possible to close the opposite end of the enclosure if it is not already otherwise so.

CLAIMS

1. A disposable reactor for use with a co-operable instrument to form a quantitative analytical device, which reactor comprises a body of capillary material, a liquid-impermeable enclosure housing the capillary body and having an opening for application of liquid to the body, at least one reagent immobilised within the enclosure, and an electrode assembly passing through the enclosure, with one portion of the assembly extending within the enclosure to engage the capillary body and another portion of the assembly extending outside the enclosure, remotely from the enclosure opening, for connection with the associated instrument.

2. A reactor according to Claim 1 wherein said enclosure and capillary body are each of elongate form with said opening at one end of the enclosure and said electrode assembly passing through the opposite end.

3. A reactor according to Claim 2 comprising a strip base for said enclosure, with said electrode assembly extending longitudinally along one side face of said base, said capillary body being also of strip form and located on said base as well as one end portion, but not the other, of said electrode assembly, and an enclosure cover extending transversely over said body and connected with said base while leaving said electrode assembly other end portion exposed.

4. A reactor according to Claim 3 wherein said electrode assembly comprises two electrodes extending in mutually spaced side-by-side manner along said base.

5. A reactor according to Claim 3 or 4 wherein said electrode assembly is directly covered with a layer of electrically insulating material except at its ends for operable engagement with said body and instrument.

6. A reactor according to any preceding claim comprising a filter located in said enclosure between said capillary body and said opening.

7. A reactor according to any preceding claim comprising a reagent immobilised within said enclosure by impregnation in said capillary body.

8. A reactor according to any preceding claim comprising a reagent immobilised within said enclosure by deposition in the interior thereof.

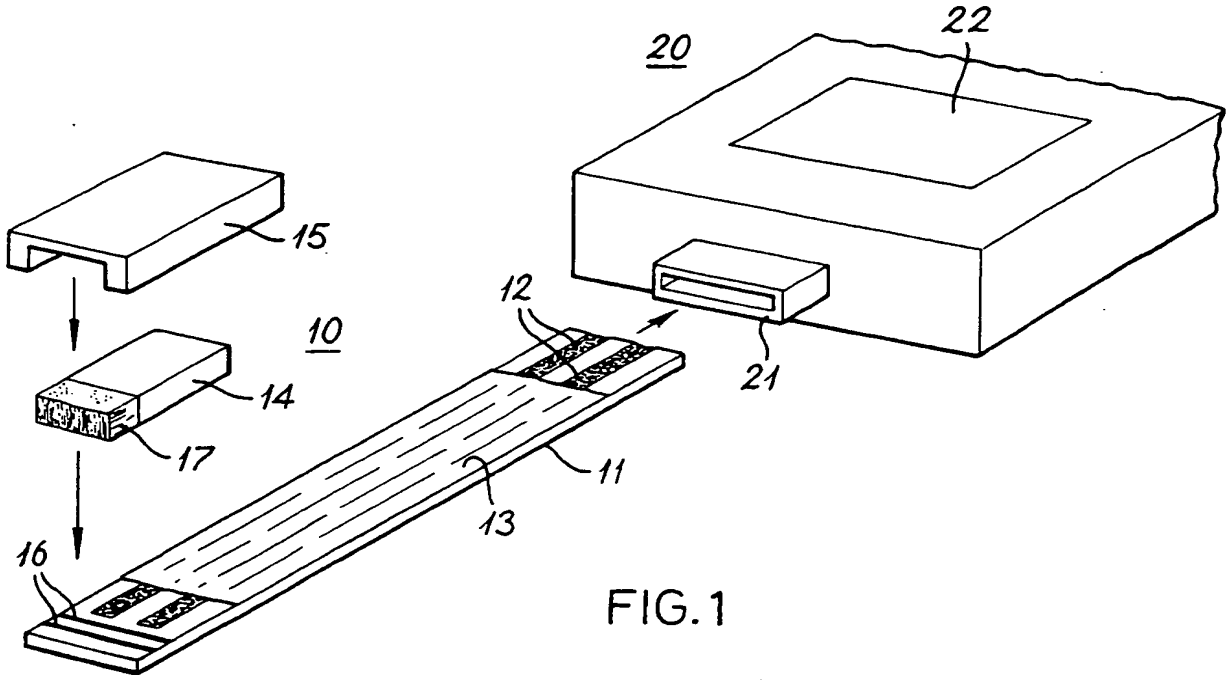
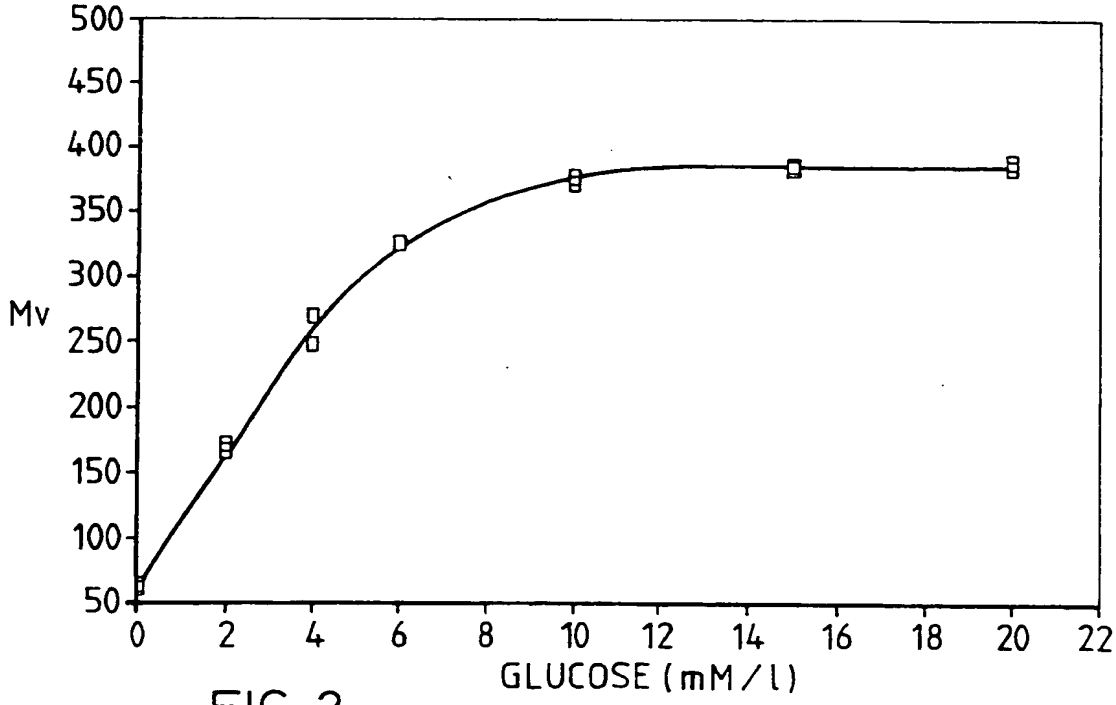

20
22
15
10
12
14
17
21
11
13
16

FIG. 1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300
250
200
150
100
50
Mv
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4
6
8
10
12
14
16
18
20
22
GLUCOSE (mM/l)

FIG. 2

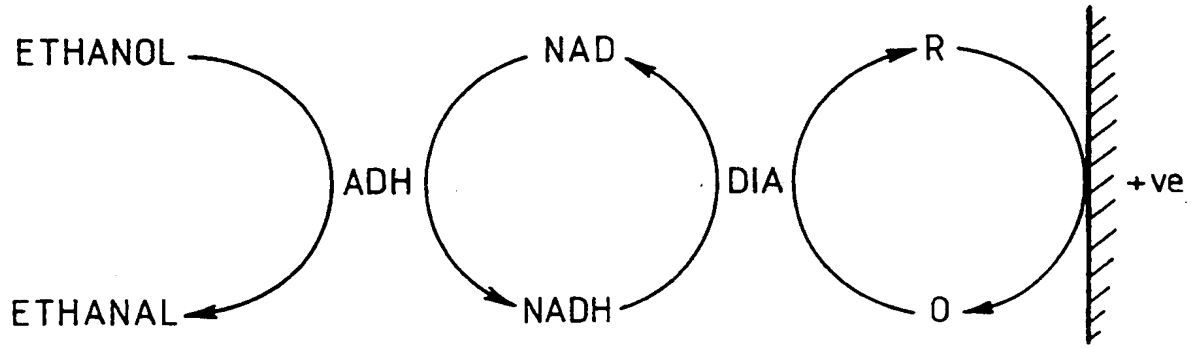
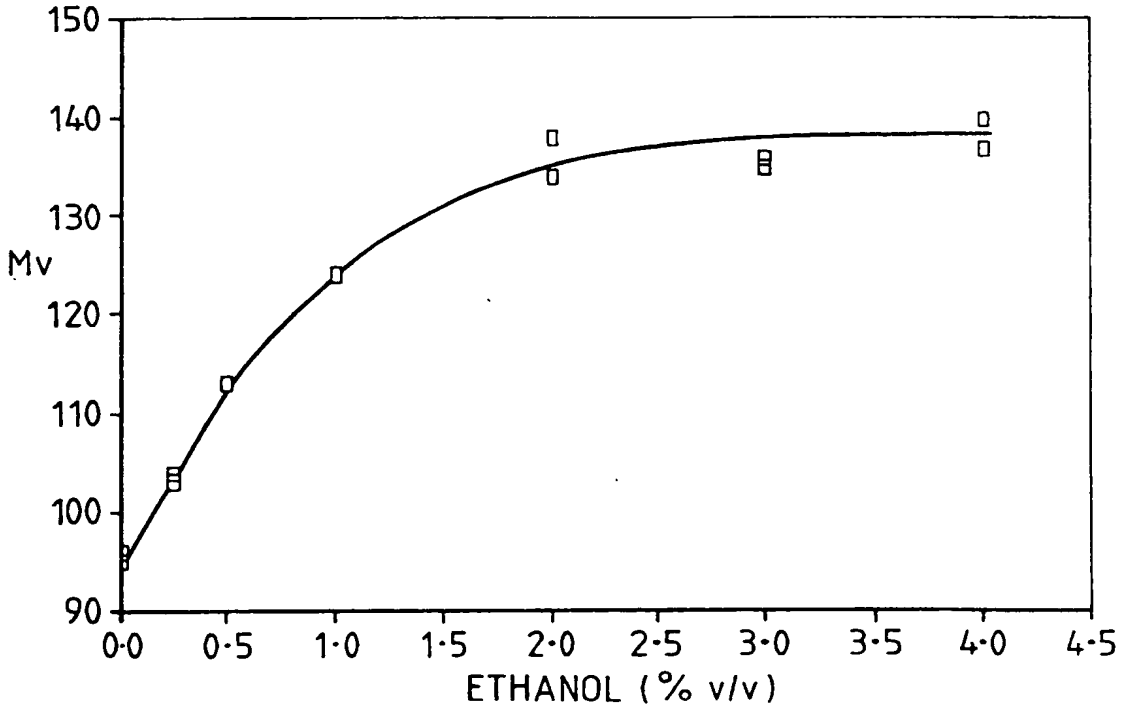

ETHANOL
ETHANAL
ADH
NAD
NADH
DIA
R
O
+ve

FIG. 3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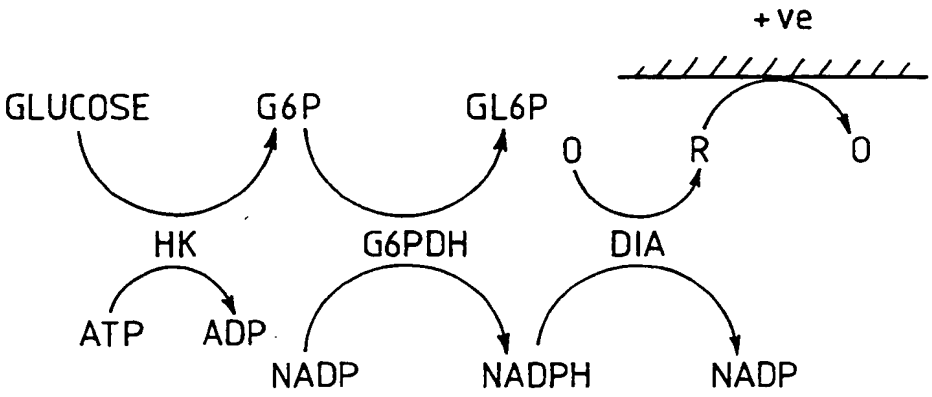
140
130
120
110
100
90
Mv
0·0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
4·0
4·5
ETHANOL (% v/v)

FIG. 4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GLUCOSE
G6P
GL6P
O
R
O
HK
G6PDH
DIA
ATP
ADP
NADP
NADPH
NADP

FIG. 5

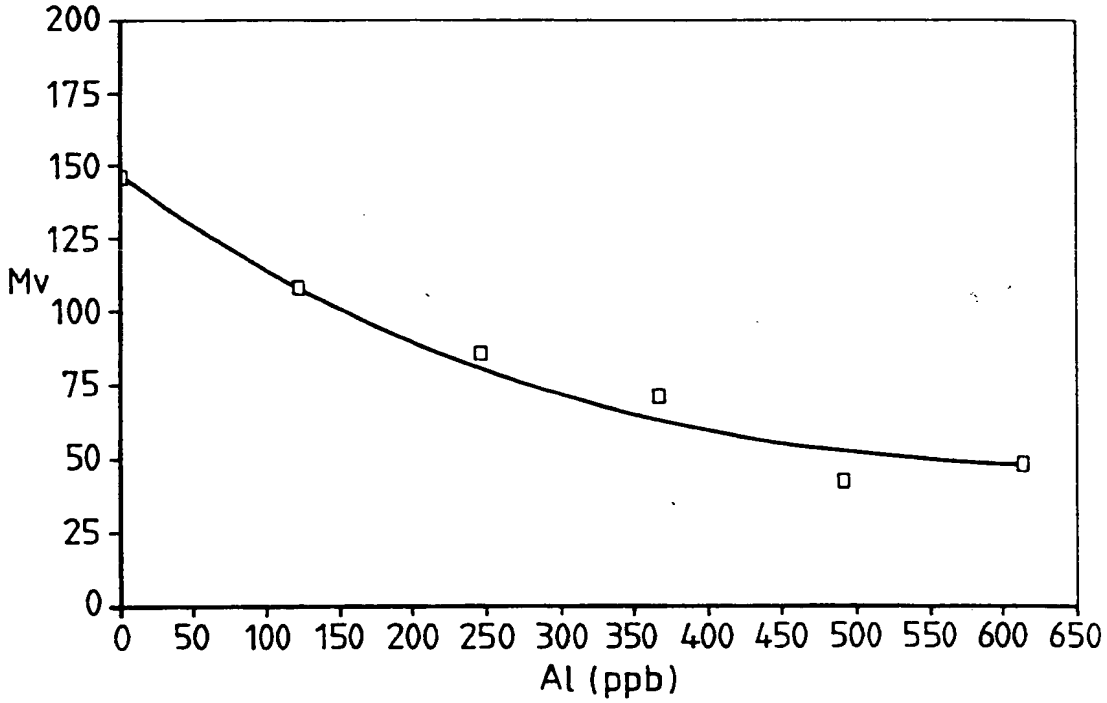
200
175
150
125
Mv
100
75
50
25
0
0 50 100 150 200 250 300 350 400 450 500 550 600 650
Al (ppb)

FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/00576

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/48; G01N27/28; B01L3/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N ; B01L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	EP,A,0 121 385 (CAMBRIDGE LIFE SCIENCES PLC) 10 October 1984 see page 11, paragraph 2 - page 14, paragraph 1; figures 1-8	1-8
Y	EP,A,0 230 472 (MATSUSHITA ELECTRIC INDUSTRIAL) 5 August 1987 see page 6, line 20 - page 8, line 6 see page 12, line 6 - page 13, line 23; figures 2-4,8,9	1-8
Y	EP,A,0 136 362 (MATSUSHITA ELECTRIC INDUSTRIAL) 10 April 1985	6
A	see the whole document	1-5,7,8
Y	EP,A,0 127 958 (GENETICS INTERNATIONAL INC) 12 December 1984	8
A	see the whole document	1-7

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21 JULY 1992	31. 07. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	R.A.P. BOSMA

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	DE,A,2 127 142 (MILES LABORATORIES INC.) 16 December 1971 see the whole document ---	1-8

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9200576 SA 57978

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		AU-A- 2775584	08-11-84
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		DE-A- 3485554	16-04-92
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		CA-A- 1218704	03-03-87
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		EP-A,B 0125137	14-11-84
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